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ORIGINAL ARTICLE

Effects of n-hexane and ethylacetate fractions of *Terminalia catappa* leaf extract in experimental *Trypanosoma brucei brucei* infection in *Wistar* rats

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Abstract

Due to the toxic effects from using conventional trypanocidal drugs, there is need for development of safer alternatives to combat trypanosomosis. The aim of this study was to evaluate the effects of n-hexane and ethylacetate fractions of *Terminalia catappa* leaf extract in *Wistar* rats experimentally infected with *Trypanosoma brucei brucei*. Forty-five (45) adult *Wistar* rats were divided into 9 groups (A–I) comprising of 5 rats each after acclimatisation for 7 days. Groups A-H were infected intraperitoneally with *T. brucei brucei* (day 0), treatment given to groups A–G at day 3 post-infection while group I were uninfected. Groups A, B and C are treated daily with 100, 200 and 300 mg/kg n-hexane fraction (HF); D, E and F treated daily with 100, 200 and 300 mg/kg ethylacetate fraction (EAF); and G treated with diminazene aceturate once. Blood was collected for parasitaemia determination and haematology. Results revealed parasitaemia detection by day 2 pi and complete clearance by day 10 pi in groups F and G, and 100% mortality in groups A and G. There was significant decrease in packed cells volume, haemoglobin concentration, red and total white blood cells in all infected groups by day 3 pi. Following treatment at day 10 pi, these haematological parameters significantly increase towards pre-infection values and were significantly higher in groups F and G compared to the other treatment groups. The ethylacetate fraction of *Terminalia catappa* leaf extract at 300 mg/kg exhibited in vivo trypanocidal activity similar to diminazene aceturate.

Keywords Trypanosomosis · *Terminalia catappa* · n-Hexane · Ethylacetate · Trypanocide

Introduction

Blood parasites constitute severe problem to livestock production and these parasites include the causative agents of babesiosis and trypanosomosis (Hashem et al. 2018). Trypanosomosis is an endemic disease affecting both humans and animals (Swallow, 2000; Ezeani et al. 2008; Mbaya et al. 2012). In Africa, the disease is caused by *Trypanosoma brucei gambiense* and *T. brucei rhodesiense* referred to as human African trypanosomosis (HAT) or sleeping sickness transmitted by tsetse flies (Mbaya et al. 2012; Radwanska et al. 2018). American trypanosomosis also referred to as Chagas' disease is caused by *Trypanosoma cruzi* and transmitted by reduviid bugs (Echeverria and Morillo 2019). The *Trypanosoma* species that commonly affect animals include *T. brucei brucei*, *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and *Trypanosoma equiperdum* (Radwanska et al. 2018; *Büscher* et al. 2019).

Trypanosomosis has continued to adversely affect economic and social well-being of sub-Saharan Africans causing great losses in livestock production (Muhanguzi et al. 2017). The disease is characterised by intermittent fever, anaemia, occasional diarrhoea, rapid loss of condition and death (Dinsa et al. 2013; Radwanska et al. 2018). Survivals usually remain

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infected for several months or years serving as reservoirs with low level of fluctuating parasitaemia (Mehlitz and Molyneux 2019). Despite efforts channelled towards chemotherapy and control of tsetse flies, the disease still pose danger threatening food security (Muhanguzi et al. 2017).

The treatment of trypanosomosis is faced with challenges including late stage of the disease, nervous system involvement and drug toxicity (Melaku and Birasa 2013; Giordani et al. 2016). Berenil (diminazene aceturate), being the most widely used drug for the treatment of animal trypanosomiasis, has been reported to be highly toxic, presented with brain damage, quivering and restlessness (Kellner et al. 1985; Melaku and Birasa 2013).

Plants have been reported to possess therapeutic effects as demonstrated in several studies. These include ameliorate effect of *Echinacea purpurea* on changes induced by *Escherichia coli* in broiler chickens (Abdallah et al. 2020a), protective effects of *Thymus vulgaris* and *Curcuma longa* on thermally oxidised oil-induced alterations in rabbits (Abdallah et al. 2020b, 2020c), potential benefits of *Chlorella vulgaris*, β-glucan, sodium alginate and *Morus alba* in *Oreochromis niloticus* and *Clarias gariepinus* (Mahmoud et al. 2020; Neamat-Allah et al. 2020a, 2020b, 2020c, 2020d).

The use of plants in the effective treatment of parasitic diseases such as trypanosomosis has been adopted by local herdsmen. *Terminalia catappa* has been reported to possess medicinal properties against bacterial, fungal and parasitic diseases (Inabo and Fathuddin 2011; Terças et al. 2017). So, in order to avoid the damages produced by conventional trypanocidal drugs, there is need to seek for safer alternatives in medicinal plants such as *Terminalia catappa*. Hence, the aim of this study was to evaluate the effects of n-hexane and ethylacetate fractions of *Terminalia catappa* leaf extract in *Wistar* rats experimentally infected with *Trypanosoma brucei brucei*.

Materials and methods

Ethics statement

This study involved the use of animals in accordance with internationally accepted guidelines. Ethical approval was granted by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

Plant collection, extract preparation and fractionation

The leaves of *Terminalia catappa* used in this study were collected within Zaria, identified and deposited with 1556 as voucher number. Extract of the leaves was prepared by drying at room temperature for 3 weeks, grinding, soaking in ethanol and washing with same solvent. After soaking and washing, solvent was evaporated using rotary evaporator. Fractions of the extract were prepared using n-hexane and ethylacetate as solvents. Lethal dose-50 (LD_{50}) of the fractions was determined using the method described by Lorke (1983) to be over 1000 mg/kg.

Trypanosome parasite

The *Trypanosoma brucei brucei* used in this study was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The parasite was continually passaged in mice to be maintained in the laboratory. Animals were infected by intraperitoneal administration of *T. brucei brucei*-infected blood containing 10^6 trypanosomes/ml, parasitaemia-monitored and treatment-administered at day 3 post-infection (pi).

Experimental design

Forty-five (45) adult *Wistar* rats were acclimatised for 7 days and divided into 9 groups (A–I) comprising 5 rats each. Rats in groups A, B and C were treated daily with 100 mg/kg, 200 mg/kg and 300 mg/kg n-hexane fraction (HF), respectively; D, E and F were treated daily with 100 mg/kg, 200 mg/kg and 300 mg/kg ethylacetate fraction (EAF), respectively; G treated once with diminazene aceturate; H were infected and untreated, while rats in group I were uninfected.

Blood collection

Blood was collected at pre-infection (day 0) and post-infection (days 2, 3, 5, 7, 9 and 10) from all rats into EDTA-coated sample tubes and processed for analysis (Neamat-Allah 2015; Neamat-Allah and El-Damaty 2016; Neamat-Allah and Mahmoud 2019). The haematological parameters determined were packed cells volume, haemoglobin concentration, red and total white blood cells counts as described by Schalm et al. (1975) and Dacie and Lewis (1991).

Data analysis

Values obtained were expressed as mean ± SEM and presented using charts. Statistical analysis of date was achieved by two-way analysis of variance (ANOVA) with *Bonferroni* multiple comparison test, using Graph Pad Prism version 5.0 for windows (Graph Pad Software, San Diego, CA, USA). Values of $P \leq 0.05$ were considered significant.

Results

Parasitaemia

Parasitaemia became patent from day 2 post-infection (pi) in all infected groups (Fig. 1), and 100% mortality recorded at day 7 pi (infected untreated) and day 9 pi (100 mg/kg HF). Following treatment, there was complete parasitaemia clearance from day 7 pi (diminazene aceturate) and day 9 pi (300 mg/kg EAF) but with persistence of parasitaemia up to day 10 pi in other treatment groups (Fig. 1).

Packed cells volume

No significant ($P > 0.05$) difference existed for packed cells volume (PCV) in all groups of rats pre-infection (Fig. 2). There was significant ($P < 0.05$) decrease in PCV in all infected groups at day 3 pi. Following treatment, PCV was significantly ($P < 0.05$) increased in groups VI (51.33 ± 0.88%) and VII (50.00 ± 1.50%), and showed no significant ($P > 0.05$) differences with those of group IX (51.50 ± 1.50%), but with significant ($P < 0.05$) differences compared to other treatment groups at day 10 pi (Fig. 2).

Haemoglobin concentration

Haemoglobin concentration (HGB) showed no significant ($P > 0.05$) difference in all groups of rats pre-infection but decreased significantly ($P < 0.05$) in all infected groups at day 3 pi (Fig. 3). At day 10 pi following treatment, HGB was significantly ($P < 0.05$) increased in groups VI (15.00 ± 0.06 g/dl), VII (14.85 ± 0.05 g/dl) and IX (15.20 ± 0.20 g/dl), with no significant ($P > 0.05$) differences between them, but significantly ($P < 0.05$) higher compared to the other treatment groups (Fig. 3).

Red blood cells count

There was no significant ($P > 0.05$) difference in red blood cells (RBC) count in all groups of rats at day 0 but this decreased ($P < 0.05$) significantly in all infected groups up to day 3 pi (Fig. 4). Following treatment, RBC count increased significantly ($P < 0.05$) up to day 10 pi but significantly ($P < 0.05$) higher in groups VI ($7.87 \pm 0.19 \times 10^6$/μl) and VII ($8.25 \pm 0.15 \times 10^6$/μl) compared to the other treatment groups (Fig. 4).

Total white blood cells

Total white blood cells (TWBC) showed no significant ($P > 0.05$) difference pre-infection in all groups of rat, but decreased ($P < 0.05$) significantly in infected groups from day 2 pi till day 5 pi. This was followed by significant ($P < 0.05$) increase up to day 10 pi with no significant ($P > 0.05$) difference in groups VI ($14.63 \pm 0.07 \times 10^3$/μl) and VII ($15.05 \pm 0.15 \times 10^3$/μl), but significantly ($P < 0.05$) higher compared to other treatment groups (Fig. 5).

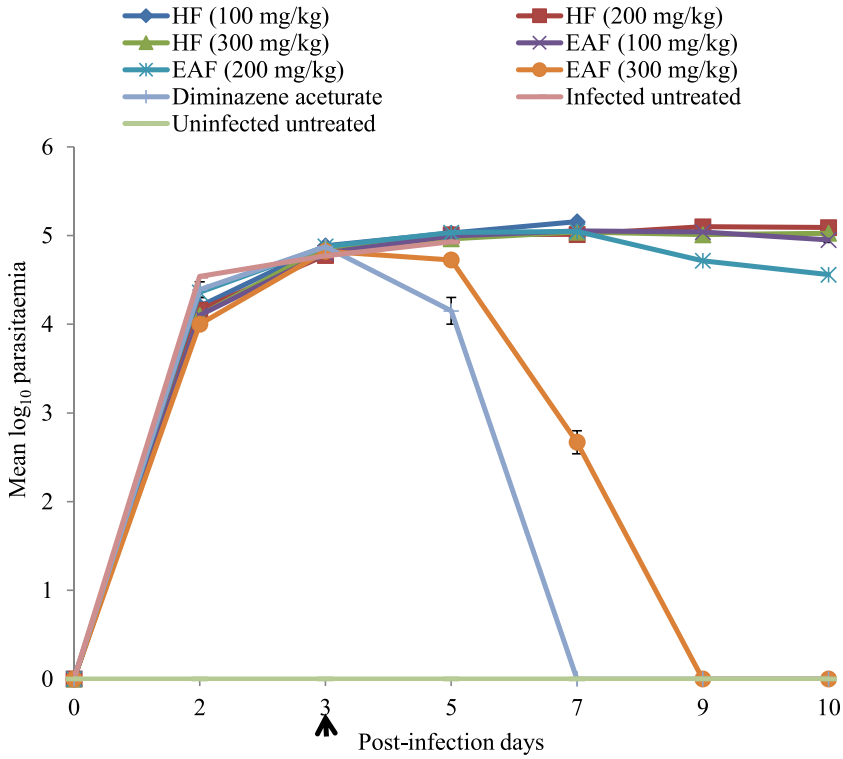

Fig. 1 Mean Log_{10} parasitaemia of *T. b. brucei*-infected *Wistar* rats administered n-hexane (HF) and ethylacetate (EAF) fractions of *Terminalia catappa* leaf extract from day 3 post-infection

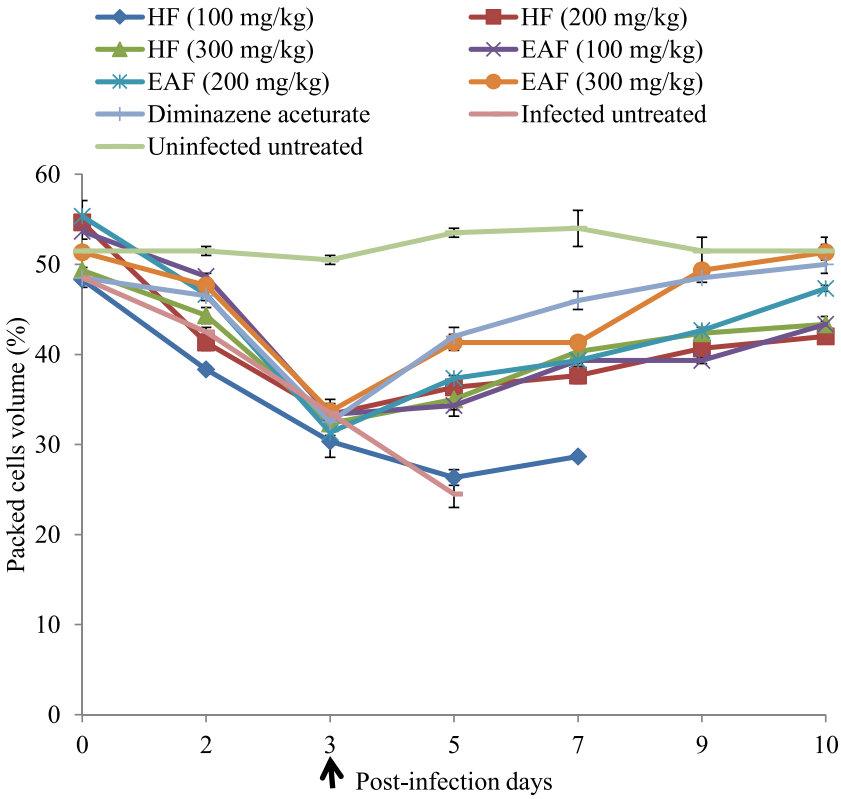

Fig. 2 Packed cells volume of *T. b. brucei*-infected *Wistar* rats administered n-hexane (HF) and ethylacetate (EAF) fractions of *Terminalia catappa* leaf extract from day 3 post-infection

Discussion

In this study, parasitaemia due to *T. brucei brucei* was detected by day 2 post-infection (pi) in infected groups (A–H). However, *T. brucei brucei* parasitaemia has been detected by day 4 pi in the reports of studies in *Wistar* rats (Umar et al. 2000, 2007; Kobo et al. 2014; Ibrahim et al. 2016; Erin et al. 2019). The detection of parasitaemia suggests potential

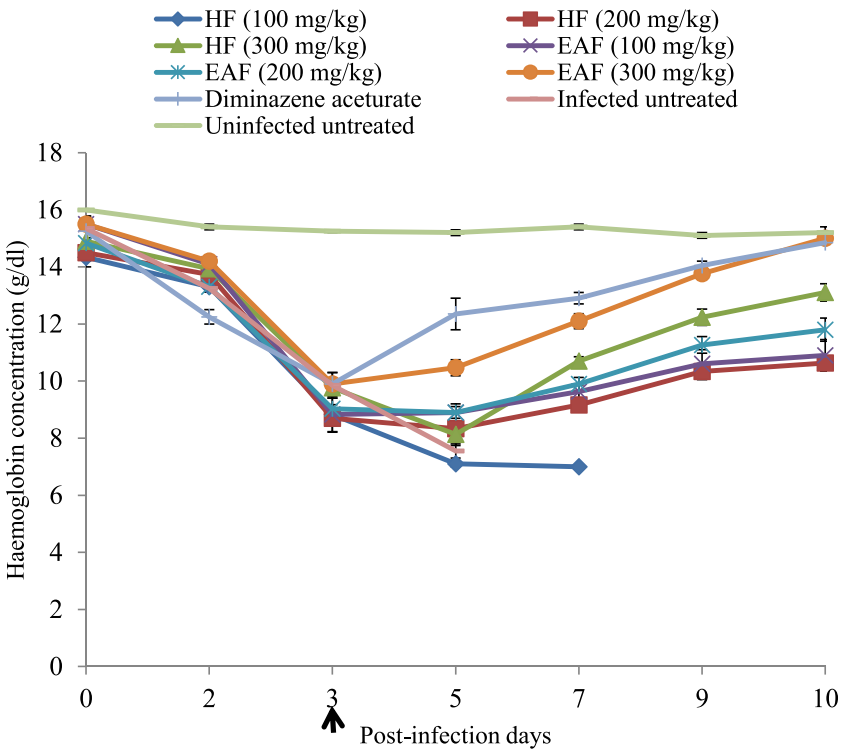

Fig. 3 Haemoglobin concentration of *T. b. brucei*-infected *Wistar* rats administered n-hexane (HF) and ethylacetate (EAF) fractions of *Terminalia catappa* leaf extract from day 3 post-infection

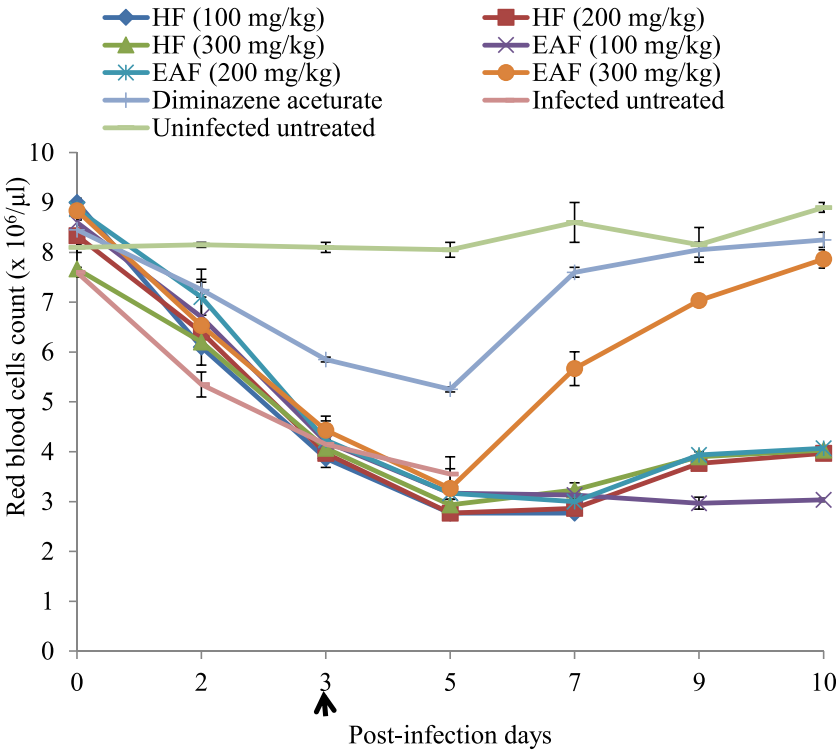

Fig. 4 Red blood cells count of *T. b. brucei*-infected *Wistar* rats administered n-hexane (HF) and ethylacetate (EAF) fractions of *Terminalia catappa* leaf extract from day 3 post-infection

parasite replication following infection. The differences in day of parasitaemia detection could have resulted from variation in ages and weights of rats, feed, season and virulence of the parasite. Also, there was anaemia and leukopaenia in infected groups depicted by the significant decrease in haematological parameters post-infection. Anaemia has been reported to be a consistent finding in trypanosomosis resulting from trypanosome flagella lashing action, undulating pyrexia, immune complexes, trypanosome toxins and metabolites, lipid peroxidation and malnutrition (Murray and Morrison 1978; Morrison et al. 1981; Saror 1982; Igbokwe 1994; Mbaya et al. 2012). The apoptosis of leucocytes and bone marrow

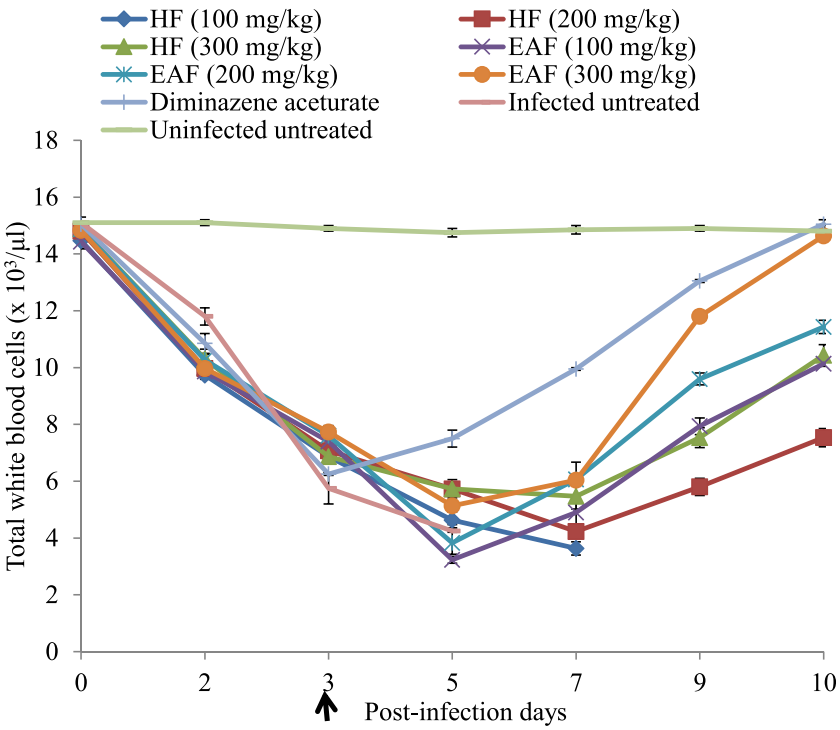

Fig. 5 Total white blood cells count of *T. b. brucei*-infected *Wistar* rats administered n-hexane (HF) and ethylacetate (EAF) fractions of *Terminalia catappa* leaf extract from day 3 post-infection

hypoplasia has been suggested to be responsible for the leukopaenia observed in trypanosomosis (Igbokwe and Anosa 1989; Marcondes et al. 2000; Kagira et al. 2006). The LD_{50} of *Terminalia catappa* fractions being greater than 1000 mg/kg suggests safety of the plant for use hence, necessitated the dose levels (100, 200 and 300 mg/kg) used in this study.

Following treatment, there was complete clearance of parasitaemia in groups treated with diminazene aceturate (group G) and 300 mg/kg ethylacetate fraction (group F) of *Terminalia catappa* leaves. Packed cells volume, haemoglobin concentration, red and total white blood cells were significantly ($P < 0.05$) increased in groups B–G but the increase was significantly ($P < 0.05$) higher in groups F and G compared to the other treatment groups at day 10 pi. Also, mortality was observed in group A despite receiving treatment at 100 mg/kg HF. The mortality could be due to low amount of trypanocidal components at this dose. However, in groups B, C, D and E, no mortality was recorded but parasitaemia was persistent in the face of increasing haematological parameters. This suggests that at these doses, trypanocidal level of the fractions was not achieved to completely clear the parasite from the blood. Rapid metabolism of the active components could possibly be responsible for the decreased trypanocidal activity observed at these lower doses. The ability of EAF at 300 mg/kg to completely clear the parasitaemia accompanied by higher haematological parameters at day 10 pi compared to HF at same dose suggests that EAF had more trypanocidal principles of importance in trypanosomosis. Antitrypanosomal activity by EAF of *Punica granatum* against *T. b. brucei* has also been reported (Inabo and Fathuddin 2011) suggesting presence of more trypanocidal constituents in EAF.

The antitrypanosomal activity of *Terminalia catappa* leaf fractions in this study evidenced by clearance of parasitaemia and restoration of haematological changes could be due to the presence of substances that act directly on the parasite, interfere with parasite replication, protect blood cell components and/or enhance the immune system of the host. These substances include flavonoids, terpenes and tannins (Shuaibu et al. 2008). Possible mechanisms involved erythrocytes protection from haemolysis via scavenging of reactive oxygen species (Umar et al. 2007; Ajakaiye et al. 2013), neutralisation of trypanosomes toxic metabolites and bone marrow stimulation (Ogoti et al. 2009; Mergia et al. 2014).

The significant clearance of parasitaemia and restoration of trypanosome-induced haematological changes by 300 mg/kg EAF in this study could be due to presence of optimum amount of constituents with antitrypanosomal activities at this dose. It could also results from the constituents acting individually or synergistically to produce these effects. The in vivo antitrypanosomal effect exhibited by *Terminalia catappa* is not uncommon as other reports have clearly demonstrated potent antitrypanosomal activity by different families of plants (Nok et al. 1993; Agbedahunsi et al. 2006; Bala et al. 2009; Inabo and Fathuddin 2011). The specific mechanisms by which antitrypanosomal activity is achieved by *Terminalia catappa* are therefore unknown and require further investigation. Hence, studies are recommended to isolate the active antitrypanosomal principles from ethylacetate fractions of *Terminalia catappa* leaves as an impetus for safer trypanocidal drugs development.

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Compliance with ethical standards

Ethical approval The use of animals in this study was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

Conflict of interest The authors declare that there is no conflict of interest.

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